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Interaction between netropsin and double-stranded DNA in capillary zone electrophoresis and affinity capillary electrophoresis

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Abstract

Capillary zone electrophoresis (CZE) and affinity capillary electrophoresis (ACE) were applied to study the interaction between netropsin and a 14mer double-stranded DNA (dsDNA). The use of a polyacrylamide coated capillary can suppress the electroosmotic flow (EOF) and the adsorption of DNA onto the wall. Better analysis of the DNA was achieved in a coated capillary upon Tris–acetate. In CZE, the peak width broadened due to the affinity interaction between dsDNA and netropsin. In ACE, *o*-toluic acid, a negatively charged molecule was used as the indicator to monitor the changes of EOF when netropsin was added to the running buffer. The 14mer dsDNA showed different mobilities upon various concentrations of netropsin due to the affinity interaction between the dsDNA and netropsin. The binding constants of this interaction were $(1.07\pm0.10)\cdot10^{5}\ M^{-1}$ calculated from CZE and $(4.75\pm0.30)\cdot10^{4}\ M^{-1}$ from ACE using a Scatchard plot. The binding stoichiometry was 1:1 calculated from CZE which was superior to ACE in this study.

Keywords: Affinity capillary electrophoresis; DNA; Netropsin

1. Introduction

DNA molecules are polymorphic, and exist in a variety of structural forms that might provide unique binding sites for small molecules [1]. Many small molecules that interact with DNA have potential therapeutic applications in targeting tumor growth, and viral, bacterial, fungal and parasitic infections, so DNA–drug interactions are a particularly important class of intermolecular interactions. The drugs targeting DNA can be divided into two categories: intercalator and minor groove binder [2]. Despite the relatively large number of DNA-binding drugs currently used in the clinic, selective toxicity towards disease-affected tissue (particularly tumors) remains an elusive goal. Furthermore, the exact mode of action for many of these compounds is poorly defined. As a result there is a major research effort, which seeks to gain a deeper insight into the molecular basis of interactions between small molecules and DNA with special emphasis on the site, mode, sequence and structural specificity of their binding reactions [3]. Though many methods such as nuclear magnetic resonance (NMR) [4], Fourier transform (FT) IR [5], Raman [6], mass spectrometry (MS) [7], X-ray [8], fluorescence [9], UV [10], cyclic voltammetry [10], T_m (melting temperature) shift [11] and equilibrium dialysis [11], etc., have been performed to study the basis of DNA–

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drug interactions, they are tedious, time-consuming and a large sample size is required. Introduction of new methods which are of low sample consumption and short analysis time is favorable for promoting the study of DNA–drug interactions.

Capillary electrophoresis (CE) has become a robust separation technique in the past few years owing to its several advantages such as short analysis time, low sample size requirement, high efficiency and flexible applications [12], and, additionally, CE can provide physiological or near-physiological buffer conditions in the analysis of biomolecules. CE is based on the mobilities of charged species through a buffer-filled capillary upon the application of a voltage. The idea of characterizing and using affinity interactions in electrophoresis is more than 50 years old, but the CE format has brought much higher versatility and precision than was previously possible [13]. CE is convenient for conducting affinity binding assays by monitoring the changes of the electrophoretic signals of the free components and/or the complexes. Many biomolecular systems such as protein–protein, protein–DNA, protein–drug, protein–sugar, DNA–peptide, peptide–drug, peptide–peptide, antibody–antigen, peptide–carbohydrate, peptide–dye and carbohydrate–drug have been studied using CE techniques [14]. Five CE methods are used for the characterization of binding interactions [15]: (1) affinity CE (ACE); (2) preequilibration CE (the same as capillary zone electrophoresis, CZE); (3) Hummel–Dreyer principle; (4) frontal analysis CE; and (5) vacancy peak analysis. Among these methods, CZE is the most appropriate for the study of molecular interactions in cases where only very small amounts of samples are available and ACE is the only method that can investigate molecular interactions quantitatively by monitoring the mobility shift of the analyte. Because the ACE method is derived from gel electrophoresis [16]: ligand (or receptor) is added into the running buffer to conduct mobility shift of receptor (or ligand). UV or laser-induced fluorescence (LIF) detection coupled with CE has been applied to the studies of protein–DNA interactions. Though the UV method has a lower sensitivity than the LIF method, it is necessary to use a fluorigenic labeling technique in the latter method, which may cause disturbances at the binding sites of the biomolecules. The UV method can provide interaction information on ''real'' molecules because no derivatization reaction is needed.

To our knowledge, there are only a few reports concerning the research of DNA–drug interactions and even quantitative determination of the binding constants using CE [17–20]. Small duplex oligonucleotides can be used as appropriate molecules for the investigation of binding properties. In the present work, we explored the interaction between a 14mer double-stranded (ds) DNA with a sequence of 5′-CCCCCTATACCCGC-3′ and netropsin (a minor groove DNA binder) by CZE and ACE in a polyacrylamide coated capillary. The peak height (in CZE) and mobility shift (in ACE) of dsDNA were monitored to get practical data. The binding constant and stoichiometry were obtained based on Scatchard analysis, and, by comparing the results obtained by CZE and ACE, the CZE method is shown to be more effective than the ACE method in probing DNA–drug interaction.

2. Experimental

2.1. Materials

All chemicals were analytical grade unless otherwise indicated. γ-Methacryloxypropyltrimethoxysilane (MAPS), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) and netropsin dihydrochloride (see Fig. 1) from *Streptomyces netropsis* were from Sigma (St. Louis, MO, USA). Tris base (ultrapure) was from Gibco-BRL (Beijing, China). Acetic acid and sodium chloride were from Shenyang Chemicals (Shenyang, China). *o*-Toluic acid (OTA) was from Shanghai Chemicals (Shanghai, China). Redistilled water was used through this work. Netropsin was

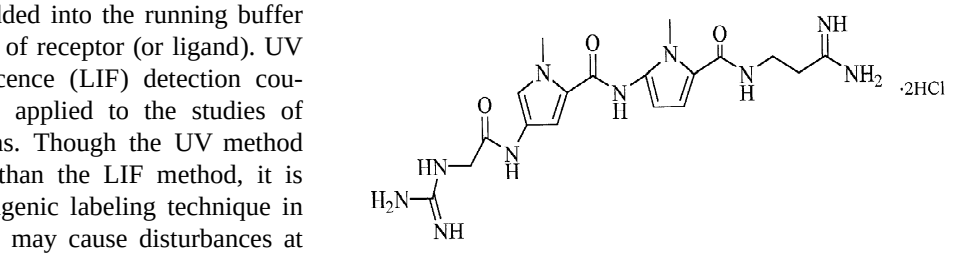

Fig. 1. The structure of netropsin.

dissolved in Tris–acetate buffer (20 m*M* Tris, 10 m*M* NaCl, the pH was adjusted to 7.2 by acetic acid) at various concentrations. The concentration of netropsin was calculated from the absorbance at 296 nm with a coefficient of 20 200 M^{-1} cm^{-1} [19]. These netropsin buffers were used as running buffers.

For the preparation of the 14mer dsDNA two complementary single-stranded (ss) DNAs having the sequences 5′-CCCCCTATACCCGC-3′ and 5′-GCGGGTATAGGGGG-3′ were purchased from TaKaRa Biotechnology (Dalian, China). Each of the ssDNAs were dissolved in Tris buffer (10 m*M* Tris, the pH was adjusted to 7.2 by acetic acid) at a concentration of 450 μ*M*. Equal aliquots of the ssDNAs (20 μl) were mixed in a centrifugal tube, and then NaCl and water were added to a total volume of 50 μl with a concentration of 50 m*M* NaCl. The presence of some salts is necessary for the oligos to hybridize. The surface of the mixture was covered with 20 μl liquid paraffin. The tube was placed in a 94 °C water bath and gradually cooled down to room temperature. Then a 14mer dsDNA was obtained. In CE procedures, sample stacking is a common phenomenon. The sample analytes will enhance electrophoretic mobilities in a lower conductive environment [21]. But if the sample has a higher ionic strength than the running buffer, the signals of analytes will decrease, and, correspondingly, the migration time will change. We have tested this phenomenon in CE of DNA and found that the NaCl concentration could not be higher than 20 m*M* in the applied Tris–acetate buffer (20 m*M* Tris, 10 m*M* NaCl, pH 7.2) to get practical data. So, the DNA samples should be desalted after annealing to obtain a lower NaCl concentration. We conducted the desalting by gel filtration on a laboratory-made Sephadex G-25 (Pharmacia) column and found that most of the NaCl was removed [22]. The final concentration of the dsDNA was measured by absorbance at 260 nm.

2.2. Apparatus

In our present work, a P/ACE MDQ system (Beckman, Fullerton, CA, USA) with a photodiode array detector and a capillary tube (Yongnian Optical Fibre Corp., Hebei, China) with an internal diameter of 50 μm were used. In order to suppress the electroosmotic flow (EOF) and the adsorption of DNA to the capillary wall, the capillary was coated with polyacrylamide by the following process:

(a) An untreated capillary was rinsed with the cycle: 1 mol/l NaOH, water, 1 mol/l HCl, water and finally with methanol.

(b) After injecting a methanol solution of MAPS, both ends of the capillary were sealed, then kept at room temperature for several hours. The capillary was then rinsed with methanol and water.

(c) A solution containing 3–4% acrylamide and constant amounts of ammonium peroxodisulfate and TEMED was prepared and stored for reaction at room temperature for 1.5 h, then followed by rinsing with water and finally purging with nitrogen gas.

The total and effective lengths of the capillary were 31.2 and 21 cm, respectively. Data were collected and processed with Beckman System software.

2.3. Procedures

For CZE, the conditions were as follows: the temperature of the cartridge and sample room was 25 °C. The capillary was filled with blank buffer (20 m*M* Tris, 10 m*M* NaCl, pH 7.2). Samples containing the mixtures of dsDNA and netropsin were injected using the pressure injection mode at 0.5 p.s.i. for 4 s (1 p.s.i.=6894.76 Pa). The applied voltage was −8 kV and the detection wavelength was 260 nm. After each run, the capillary was rinsed with water for 1 min at 20 p.s.i. Each concentration was run in duplicate.

For ACE, the conditions were as follows: the temperature of the cartridge and sample room were 25 °C. The capillary was filled with running buffer containing netropsin by pressure. Samples of 1.4 μ*M* dsDNA were injected using the pressure injection mode at 0.4 p.s.i. for 4 s. The applied voltage was −9 kV and the detection wavelength was 260 nm. After each run, the capillary was rinsed with water for 1 min at 20 p.s.i.

2.4. Quantitative models of the binding assays

In the binding studies, the binding constants are important parameters. Scatchard analysis is a com-

mon way to linearize the binding data, and the model can be expressed as Eq. (1):

$$\frac{r}{C_f} = -Kr + nK \quad (1)$$

where r is the ratio of concentrations of the bound ligand (or receptor) to total receptor (or ligand) and C_f is the unbound ligand (or receptor) concentration. K is the apparent binding constant and n is the number of binding sites [23]. The Scatchard-type method is valid only for binding systems displaying moderate to high association [24]. In the CZE section of this work, r is the ratio of concentrations of the bound DNA to total netropsin in the sample.

In ACE, if we define μ_0^{ep} as the receptor's electrophoretic mobility in blank buffer and μ_t^{ep} as its electrophoretic mobility in the buffer containing ligand at a certain concentration, then the expression $\Delta\mu^{ep} = \mu_t^{ep} - \mu_0^{ep}$ shows the mobility change of the receptor when the running buffer is added with a certain concentration of ligand. $\Delta\mu^{ep}$ is as a function of [L], the ligand concentration in the running buffer. Then Scatchard equation can be converted to Eq. (2):

$$\frac{\Delta\mu^{ep}}{[L]} = -K\Delta\mu^{ep} + K\Delta\mu_{max}^{ep} \quad (2)$$

where $\Delta\mu_{max}^{ep}$ is the mobility change in the case where netropsin is at saturate concentration ([L]>>[R]), [R] is the injected receptor concentration [25]. This model is valid only in rapid equilibration systems.

3. Results and discussion

3.1. Comparison of the coated and non-coated capillaries

Many macrobiomolecules are prone to be adsorbed electrostatically on the surface of fused-silica capillaries [25]. In general, this phenomenon is unfavorable, or even fatal for the analysis of macrobiomolecules. Though DNA molecules are less adsorptive than proteins to non-coated capillaries, pretreatment of the inner surface of the fused capillary column is critical for CE of DNA [26]. In our experiments, we compared the effects of DNA analysis in polyacrylamide-coated and non-coated capillaries (see Fig. 2). Because DNA is a highly negatively charged molecule, it can migrate readily through the coated capillary under a reverse electric field in which EOF is extremely repressed. The migration time was only 3.89 min in the coated capillary compared to 16.82 min in the non-coated capillary, and, the peak shape of DNA in coated capillary was much more symmetrical than in the non-coated capillary. Therefore, we prefer a coated capillary to a non-coated capillary in the binding assay.

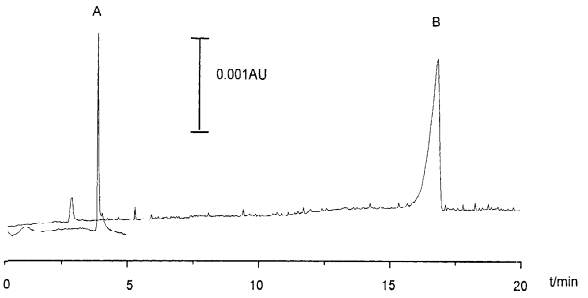

Fig. 2. Comparison of the DNA signals of 4 μ*M* DNA in an (A) coated capillary and (B) noncoated capillary. The applied voltages in A and B were −8 and 8 kV, respectively. Other running conditions were identical, see CZE conditions in the Experimental section.

3.2. The interaction between the 14mer dsDNA and netropsin in CZE

Standard samples containing the 14mer dsDNA in the range 0.5–12.0 μ*M* were injected. The peak height of each sample was proportional to the concentration of dsDNA. The relationship between peak height and dsDNA concentration could be expressed as $y = 35.4 + 484.9x$, with a coefficient of 0.998. Then the corresponding concentrations of dsDNA in the binding assay were calculated from this calibration curve.

Fig. 3 shows the electropherograms of the mixtures containing the 14mer dsDNA and netropsin and the 14mer dsDNA only. When a mixture of dsDNA and netropsin is injected, the complex no longer survives to reach the detector when we apply an electric field, because dissociation occurs on-column. This dissociation process is continuous which means that the interaction between netropsin and dsDNA

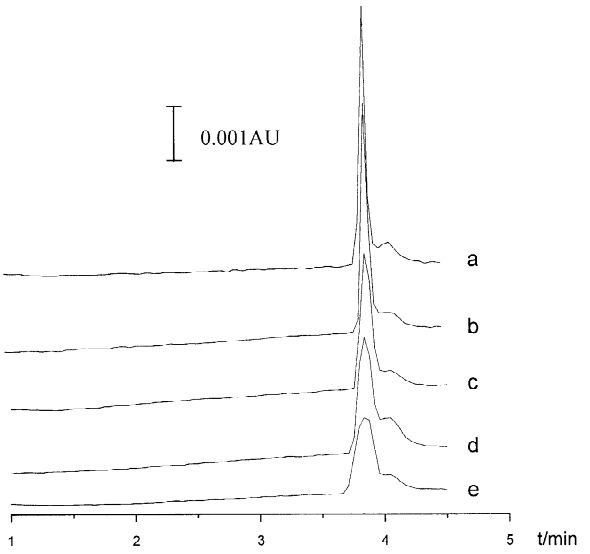

Fig. 3. Electropherograms of 14mer DNA mixed with various concentrations of netropsin. (a) 11.1 $\mu M+0$ μM netropsin; (b) 11.1 $\mu M+3$ μM netropsin; (c) 11.1 $\mu M+19.8$ μM netropsin; (d) 11.1 $\mu M+23.8$ μM netropsin; (e) 11.1 $\mu M+39.9$ μM netropsin. Conditions were as follows: injection: 0.5 p.s.i. for 4 s. Detection wavelength: 260 nm. Applied voltage: -8 kV. Capillary: polyacrylamide coated capillary of 31.2 cm (effective length 21 cm)$\times$ 50 μm I.D.

lengthens the dsDNA zone and causes the peak broadening. Accordingly, the peak height of dsDNA decreases, but the peak areas do not change, the peak heights only present the value of the original unbound dsDNA concentrations in the mixtures containing the 14mer dsDNA and netropsin. This is a common character of the fast on-and-off kinetic binding reaction [16,27]. Netropsin is positively charged at pH 7.2, so it could not migrate through the coated capillary under a reverse electric field.

It should be noted that Hamdan et al. conducted a rather different class of binding between netropsin and a 12mer oligonucleotide duplex: d(AAATTATATTAT)·d(ATAATATAATTT). They observed a remarkable signal of the complex, which was separated from free DNA [18]. Our results differed from theirs, probably because we used a different sequence and length of DNA. Apart from this aspect, they annealed the two complementary ssDNA with the presence of netropsin on column. During the annealing, netropsin most likely embeds in the duplex and conformation of the duplex may change a little from the normal B-form duplex. This process is not reversible and then the separate signal of the complex could be found in CE.

To quantitatively characterize the interaction between netropsin and the 14mer dsDNA, the concentrations of free dsDNA were calculated from the calibration curve, and thus the r values were obtained. Fig. 4 is the Scatchard plot conducted by using the CE data of various samples. K was calculated to be $(1.07\pm0.10)\cdot10^5$ M^{-1} from the slope. When the intercept was divided by the slope, a value of 1.17 for n was obtained indicating that the binding stoichiometry was 1:1.

3.3. The interaction between the 14mer dsDNA and netropsin in ACE

Netropsin was added to the running buffer to obtain different concentrations. The mobility of DNA was reflected as an average value of free DNA and DNA–netropsin complex [25]. Because netropsin is positively charged, the complex showed an apparent smaller ratio of negative charge to mass than the free DNA. So, the larger the concentration of netropsin, the larger the fraction of DNA–netropsin contributed to the total dsDNA, resulting in the longer migration time of DNA. Fig. 5 shows the representative electropherograms when netropsin was added to the running buffer with varying concen-

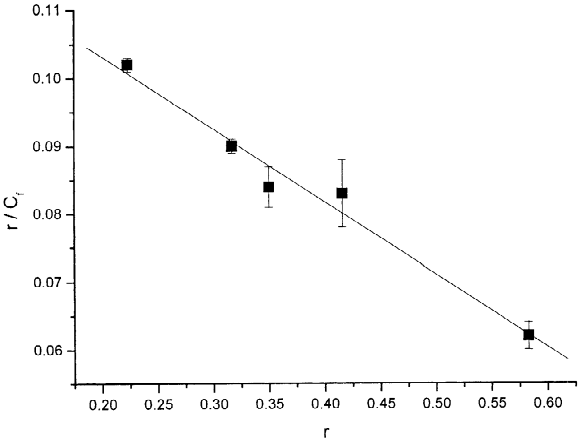

Fig. 4. Scatchard plot of the interaction between netropsin and 14mer DNA. Experimental conditions as in Fig. 3.

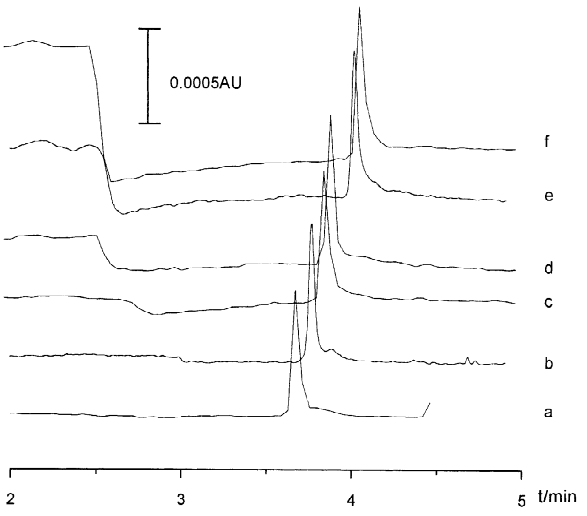

Fig. 5. Electropherograms of 1.4 μ*M* 14mer dsDNA in buffers containing different netropsin concentrations. (a) In blank buffer; (b) 5 μ*M*; (c) 12 μ*M*; (d) 20 μ*M*; (e) 50 μ*M*; (f) 75 μ*M*. Conditions were as follows: injection: 0.4 p.s.i. for 4 s. Detection wavelength: 260 nm. Applied voltage: −9 kV. Capillary: polyacrylamide coated capillary of 31.2 cm (effective length 21 cm)× 50 μm I.D.

trations and a fixed amount of dsDNA (1.4 μ*M*) was injected.

3.4. Quantitative evaluation of the interaction between netropsin and 14mer dsDNA

Some neutral markers, such as dimethylformamide, dimethylsulfoxide and mesityl oxide are often used to indicate the EOF. But these markers are not of use in case EOF is extremely small. We selected OTA (a negatively charged molecule under pH 7.2) as the indicator to monitor the changes of EOF when netropsin was added into the running buffer. Though OTA is not a neutral molecule, it does not interact with netropsin. μ^{app}, the apparent mobility of OTA, was 16.07 $cm^2\ min^{-1}\ (kV)^{-1}$ in blank buffer and 16.08 $cm^2\ min^{-1}\ (kV)^{-1}$ in 75 μ*M* netropsin buffer. Then we considered that the μ^{app} of OTA did not change when netropsin was added to the running buffer. In CE, $\Delta\mu^{ep}=\Delta\mu^{app}-\Delta\mu^{eo}$ ($\Delta\mu^{app}$ is the change of apparent mobility and $\Delta\mu^{eo}$ the change of EOF mobility). Since the μ^{app} of OTA did not change when netropsin was added to the running buffer, we considered that the term $\Delta\mu^{eo}$ was zero, which meant the EOF in blank buffer was equal to that in netropsin buffer in the range of 0–75 μ*M*. Then we can substitute $\Delta\mu^{app}$ for $\Delta\mu^{ep}$ under our applied ACE conditions, and, Eq. (2) can be re-expressed as Eq. (3):

$$\frac{\Delta\mu^{app}}{[L]} = -K\Delta\mu^{app} + K\Delta\mu^{ep}_{max} \quad (3)$$

To quantitatively characterize the interaction between netropsin and the 14mer dsDNA in ACE, Eq. (3) was used as the quantitative model. Fig. 6 is the Scatchard plot conducted using the ACE data. *K* was calculated to be $(4.75\pm0.30)\cdot10^4\ M^{-1}$. The results of *K* in CZE and ACE were a little smaller than the value obtained by Biswas et al. using CZE [19]. The difference is mainly due to the different sequences, the lengths of DNA and even between the kinds of buffer used in our work and theirs. And, it is worth noting that different CE methods may provide notable different quantitative results in the study of molecular interactions [28].

3.5. Comparison between CZE and ACE

From the results obtained by CZE and ACE, we can see that CZE showed the larger value of *K*, which was about twofold of that in ACE. The different results can be attributed to two aspects. First, the intrinsic difference in these methods: one is

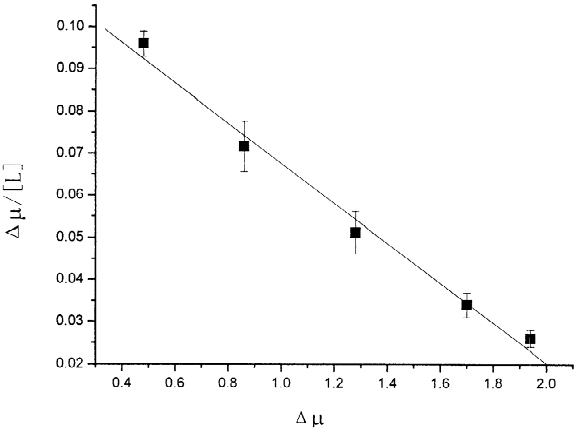

Fig. 6. Scatchard plot of the data in ACE. Experimental conditions as in Fig. 3.

quantitated by using the peak height and the other is by the migration mobility. Second, the different ranges of the ratios of receptor to ligand [28]. In addition, CZE needs smaller amounts of reagents and can also provide the binding stoichiometry.

The base specialization that makes netropsin bind preferentially to AT base pairs is provided by close Van der Waals contacts between adenine C-2 hydrogen and CH groups on the pyrrole rings of the drug molecules [29]. The molecule of netropsin is flat, crescent shaped, positively charged and has some donor or acceptor groups. These features allow it to fit tightly in the minor groove of dsDNA [30]. Besides Van der Waals force, these interactions may include hydrogen bonding, hydrophobic interactions and electrostatic interactions. In addition to the binding between base pairs and netropsin, the outside edge stacking interactions with the DNA phosphate backbone may contribute to the total binding of this interaction. DNA–netropsin binding can induce little structural rearrangement of the DNA helix to adapt the characteristic crescent shape of netropsin [3].

4. Conclusions

CZE and ACE were applied to quantitatively evaluate the interaction between a 14mer dsDNA and netropsin. Each run could be accomplished within 4.5 min. The binding constant could be obtained easily. Our results showed that CZE and ACE are powerful, sensitive, quantitative and fast methods, and can be applied to the study of interactions between DNA and drugs. CZE was superior to ACE because the former requires less amounts of reagents, and further, it can provide the binding stoichiometry. CZE and ACE can afford some useful information in the aspect of designing new ligands targeting DNA for the treatments of diseases.

Acknowledgements

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References

[1] R.R. Sinden, DNA Structure and Function, Academic Press, San Diego, CA, 1994.
[2] A.Y. Chen, C. Yu, B. Gatto, L.F. Liu, Proc. Natl. Acad. Sci. USA 90 (1993) 8131.
[3] I. Haq, J. Ladbury, J. Mol. Recognit. 13 (2000) 188.
[4] S. Pavlopoulos, W. Bicknell, G. Wickham, D.J. Craik, J. Mol. Recognit. 12 (1999) 346.
[5] J.-F. Neault, H.A. Tajmir-Riahi, J. Biol. Chem. 271 (1996) 8140.
[6] J. Stanicová, G. Fabriciová, L. Chinsky, V. Šutiak, P. Miškovský, J. Mol. Struct. 478 (1999) 129.
[7] V. Gabelica, E.D. Pauw, F. Rosu, J. Mass Spectrom. 34 (1999) 1328.
[8] O. Kennard, Pure Appl. Chem. 65 (1993) 1213.
[9] J.B. Chaires, N. Dattagupta, D.M. Crothers, Biochemistry 21 (1982) 3933.
[10] S.J. Zhang, X.Q. Lin, Chin. Chem. Lett. (Beijing) 12 (2001) 619.
[11] J. Ren, J.B. Chaires, Biochemistry 38 (1999) 16067.
[12] B.L. Karger, in: J.P. Landers (Ed.), Handbook of Capillary Electrophoresis, CRC Press, Boca Raton, FL, 1994, Foreword.
[13] N.H.H. Heegaard, Electrophoresis 23 (No. 6) (2002), Preface.
[14] F.A. Gomez, J.N. Mirkovich, V.M. Dominguez, K.W. Liu, D.M. Macias, J. Chromatogr. A 727 (1996) 291.
[15] N.H.H. Heegaard, R.T. Kennedy, Electrophoresis 20 (1999) 3122.
[16] K. Shimura, K. Kasai, Anal. Biochem. 251 (1997) 1.
[17] C. Li, L.M. Martin, Anal. Biochem. 263 (1998) 72.
[18] I.I. Hamdan, G.G. Skellern, R.D. Waigh, Nucleic Acids Res. 26 (1998) 3053.
[19] K. Biswas, S. Pal, J.D. Carbeck, D. Kahne, J. Am. Chem. Soc. 122 (2000) 8413.
[20] T.R. Battersby, D.N. Ang, P. Burgstaller, S.C. Jurczyk, M.T. Bowser, D.D. Buchanna, R.T. Kennedy, S.A. Benner, J. Am. Chem. Soc. 121 (1999) 9781.
[21] R.L. Chien, D.S. Burgi, Anal. Chem. 64 (1992) 1046.
[22] X. He, X. Zhou, B. Lin, Chin. J. Anal. Chem., in press.
[23] G. Scatchard, Ann. N. Y. Acad. Sci. 51 (1949) 660.
[24] R.S. Keyes, A.M. Bobst, Biophys. Chem. 45 (1993) 281.
[25] I.J. Colton, J.D. Carbeck, J. Rao, G.M. Whitesides, Electrophoresis 19 (1998) 367.
[26] J.P. Landers, in: Handbook of Capillary Electrophoresis, CRC Press, Boca Raton, FL, 1994, p. 138.
[27] B. Zhang, H. Xue, J.T.-F. Wong, B. Lin, Chromatographia 48 (1998) 268.
[28] J.C. Kraak, S. Busch, H. Poppe, J. Chromatogr. 608 (1992) 257.
[29] M.L. Kopka, C. Yoon, D. Goodsell, P. Pjura, R.E. Dickerson, Proc. Natl. Acad. Sci. USA 82 (1985) 1376.
[30] B.H. Geierstranger, D.E. Wemmer, Annu. Rev. Biophys. Biomol. Struct. 24 (1995) 463.